

AD _____

Award Number: DAMD17-99-1-9065

TITLE: Magnetic Resonance Studies of Photosensitizers and their
Effect in Tumors

PRINCIPAL INVESTIGATOR: Subbaraya Ramaprasad, Ph.D.

CONTRACTING ORGANIZATION: University of Nebraska Medical Center
Omaha, Nebraska 68198-5100

REPORT DATE: August 2001

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are
those of the author(s) and should not be construed as an official
Department of the Army position, policy or decision unless so
designated by other documentation.

20020814 179

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE August 2001	3. REPORT TYPE AND DATES COVERED Annual (15 Jul 99 - 14 Jul 01)	
4. TITLE AND SUBTITLE Magnetic Resonance Studies of Photosensitizers and their Effect in Tumors			5. FUNDING NUMBERS DAMD17-99-1-9065	
6. AUTHOR(S) Subbaraya Ramaprasad, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Nebraska Medical Center Omaha, Nebraska 68198-5100 E-Mail:			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words)				
14. SUBJECT TERMS Breast Cancer				15. NUMBER OF PAGES 10
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Body.....	4
Appendices.....	8

Progress Report

Title of the Project: MAGNETIC RESONANCE STUDIES OF PHOTO SENSITIZERS AND THEIR EFFECT IN TUMORS.

Award No: DAMD17-99-1-9065; P.I, Subbaraya Ramaprasad, Ph.D.

Subject: Annual report for the above award

Note: This grant was transferred to our institution only late this year (9-05-01) and hence the limited scientific progress in the short duration.

A significant part of the project is the tumor imaging, spectroscopic investigations, and detailed statistical analysis following the administration of photosensitizers and irradiation with the laser light at the appropriate wavelength. The presence of the photosensitizer in the tumor will be detected in the in vivo tumor model in mice. The growth and maintenance of cells, inoculating the cells and observe the growth pattern, and selecting the tumors of appropriate size for MR studies constitutes a small but essential step towards this goal. Following the grant transfer I have added a new part-time research assistant(technician) to perform some of the studies and help the P.I and the team with data collection, maintaining the records, and to ultimately develop into a full team member.

The basic operation of our instrument, preliminary optimization of the MR imager to record spectral data in the in vivo and in vitro state has also been pursued by the student under the supervision and guidance of the P.I.

The student has learnt many skills and knowledge in a short time. In particular, the technician has received training in animal handling, blood drawing under anesthesia, anesthetization of rodents using isoflurane and N₂O gas, or injectables such as ketamine and xylazine.

Secondly she has learnt the science and arts of maintaining the cells in exponential phase with advise from Dr. Joshi(Co-I on the grant). In particular she has practiced growing and maintaining the breast cancer cells such as SKBR3 and MRA231. Although our immediate aim is to use RIF cells in our studies , the experience

that has been gained should serve the long term objectives in a significant way. Additionally, as was suggested earlier, much will be gained by performing studies on RIF cells and the above breast cancer cells in the vitro state also.

A significant part of the stated **objectives** is the production of the required fluorinated photosensitizers in sufficiently large quantities for in vivo MR studies. Such a significant part requires exploring several routes to the synthesis of fluorinated compounds. The studies thus require optimized efficient ways to preparation of the compounds. The discoveries, accomplishments, and results of the studies during the short time requires efforts to make sensitizers with more fluorines, ways to synthesize compounds in large amounts required for the project. Such studies are being made by the co-investigator at RoswellPark Cancer Institute at Buffalo(Dr. Ravi Pandey, Ph.D). The placement of in vivo MR imager magnet operating at 7.0 T at our center(UNMC), and the compact laser system for the project signifies the presence of technological equipment required for the project. This process constitutes a significant step towards achieving the major objectives stated in the proposal.

We expect the photosensitizers to absorb light in the 630-660 nm range. The laser system we have installed from Spectra Physics with the dye laser (model 375B) unit will provide power in the range of 1-1.4 watts at the end of the fiber. We have also added a new collimator that provides the user with the flexibility to align and obtain better and uniformly intense regions of laser light intensity. Using the power meter(407A model) we have verified the power at different distances from the fiber tip. We expect this instrument to provide sufficient power for all the studies proposed in the project. Following the production of tumors in the mice, we expect to use the system for many control and PDT related studies involving many photosensitizers. We will also make some preliminary studies involving the well-known Photofrin for comparative studies.

Our immediate objective is to produce in sufficiently large quantity the fluorinated analogs in which one or more than one methyl groups are replaced with trifluoromethyl substituent(s). Unfortunately, these hematoporphyrins are unsymmetrical in nature, and thus, the related di-trifluoromethyl analogs have dispersed ^{19}F NMR signals. In symmetrical fluorinated porphyrins, the fluorines are equivalent, which results in a single, more intense NMR signal. The symmetrical fluorinated porphyrins that we will use in our studies are being synthesized in large quantity. The routes involved in the

synthesis of these type of compounds and the yield of the products are also mentioned in the discussion part related to the preparation of these compounds.

Discussion on the Synthesis of Fluorinated Photosensitizers:

The symmetrical fluorinated porphyrin, 7,18-dichloroethyl-12,13-di (ethoxy-carbonyl vinyl)- 8,17-dimethyl- 2,3-di(trifluoroethyl) porphyrin **10** was synthesized by the following "3 + 1" approach(see Appendix for the details).

2,5-Dimethyl-3,4-bis(1H,1H-trifluoroethyl-1-yl)pyrrole¹ **1** was treated with sulfuryl chloride in dry THF to give the 2,5-dicarboxy-3,4-bis(1H,1H-trifluoroethyl) derivative in 80% yield, purified by formation of the disodium salt with sodium carbonate and precipitating the product **2** by neutralizing with hydrochloric acid, mp 250-255 °C, ¹H NMR (d₆-acetone): δ 3.5 (q, 4H). A solution of the dicarboxy acid **2** was taken up in 1, 2-dichloroethane and water containing sodium bicarbonate and to this mixture was added while stirring an aqueous solution of sodium iodide and iodine. The reaction pot was then wrapped with aluminum foil and stirred at room temperature for 72 hours under nitrogen. The mixture was then treated with sodium bisulfide carefully until the color of iodine disappeared. The mixture was extracted with methylene chloride; the organic extract was washed with water, dried (MgSO₄), filtered and evaporated to give the product **3** in 81% yield as a light colored solid; TLC (CH₂Cl₂), one spot, rf 0.7, ¹H NMR (CDCl₃) δ 3.5 (q, 4H). The iodine product **3** was taken up in ethanol (95%) and treated with ammonium chloride and zinc dust, stirred at 75 °C for 18 hours (overnight), cooled, and filtered to remove zinc dust, washing the filter cake with methylene chloride. The solvent was evaporated and the residue was dissolved in minimum methylene chloride, and eluted with methylene chloride through a silica gel column; product **4** was thus obtained in 82% yield.

Benzyl 5-acetoxymethyl-4-(2-chloroethyl)-3-methyl pyrrole-carboxylate **5** was prepared according to the literature procedure². 3,5-Bis-trifluoroethyl pyrrole **4** was reacted with two equivalents of **5** in anhydrous methylene chloride in the presence of Montmorillonite clay by stirring at r.t. for 48 hours to give the corresponding tripyrrane **6**, in 25% yield. This reaction's product yield was raised to 51% by carrying out the reaction in absolute ethanol in the presence of p-toluenesulfonic acid⁴, stirring at 70 °C for 20 hours under nitrogen; ¹H NMR of **6** (CDCl₃): δ 2.25 (s, 6h, CH₃×2), 2.8 (t, 4H), 3.3 (m, 6H), 3.7 (s, 2H), 3.4 (s, 4H, -CH₂Ph), 7.0 (m, 10H, aromatic). Product **6** was hydrogenated in the presence of Pd/C in THF to give **7** as a white solid in quantitative yield; ¹H NMR of **7** (CDCl₃) indicated the absence of aromatic protons: δ 1.5 (s, 6H), 1.98 (m), 3.65 (m).

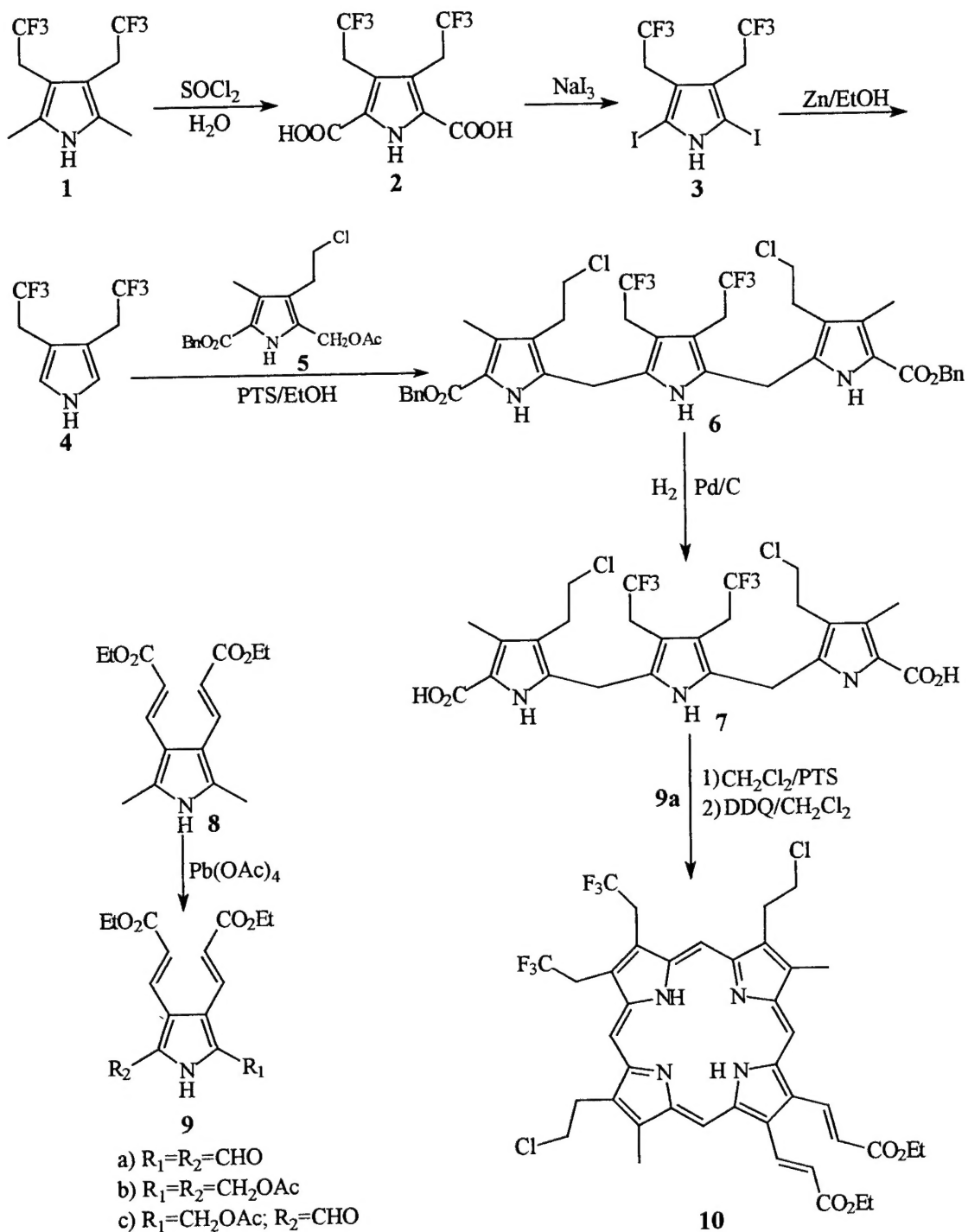
9a were synthesized by following the literature method³, although in relatively lower yield as the formation of the acetoxymethyl derivatives **9b** and **9c** could not be eliminated.

The desired porphyrin **10** was synthesized from **9a** and **7** using the "3+1" methodology; two different procedures were tried. In the first, the tripyrrane 2,5-dicarboxy derivative **7** was reacted with **9a** in the presence of trifluoroacetic acid³ in anhydrous methylene chloride under argon to give product **10** after subsequent treatment with DDQ in methylene chloride; there was, however, accompanying extensive

decomposition and the yield of **10** was very low. In the second procedure the product **10** could be isolated in low yield when this coupling reaction was carried out in the presence of p-toluenesulfonic acid in methylene chloride. Currently we are attempting to improve the yield. The proton NMR spectrum recorded on a 400 MHz instrument is enclosed with this report (see Appendix). The chemical shift of the pertinent resonance peaks in this ^1H NMR spectrum of product **10** recorded in deuterated chloroform(CDCl_3) are: δ 10 (d, 4H), 9 (2H), 6.5 (d, 2H), 4.48 (d, 2H), 4.25 (m, 4H), 4.08 (m, 2H), 2.8 (s, 4H), 1.8 (m), 1.25 (s, 6H).

As discussed, we have now established the reaction conditions for the preparation of the starting fluorinated porphyrin. Currently this compound is being prepared in larger quantity, and then will be converted to the bis-2-chloroethyl substituents into the related vinyl groups by base-catalyzed dehydrohalogenation. The corresponding vinyl porphyrin will then be reacted with HBr/acetic acid and various alkyl-alcohols (methyl-, heptyl-, decyl-) to afford the related alkyl ether analogs with variable lipophilicity. On the basis of their in vivo PDT efficacy in the mice foot tumor model, we will then select the best candidate for the detailed in vivo Magnetic resonance and biological studies proposed in the project.

APPENDIX



Scheme of 7,18-dichloroethyl-12,13-di(ethoxycarbonyl vinyl)- 8,17-dimethyl- 2,3-di(trifluoroethyl) porphyrin synthesis



¹H NMR of product **10** (CDCl₃, 400 MHz):

